Biofuels

Chemical preconversion: application of low-severity pretreatment chemistries for commoditization of lignocellulosic feedstock

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Chemical preconversion: application of low-severity pretreatment chemistries for commoditization of lignocellulosic feedstock

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Securing biofuels project financing is challenging, in part because of risks in feedstock supply. Commoditization of the feedstock and decoupling its supply from the biorefinery will promote greater economies of scale, reduce feedstock supply risk and reduce the need for overdesign of biorefinery pretreatment technologies. We present benefits and detractions of applying low-severity chemical treatments or 'chemical preconversion treatments' to enable this approach through feedstock modification and densification early in the supply chain. General structural modifications to biomass that support cost-effective densification and transportation are presented, followed by available chemistries to achieve these modifications with minimal yield loss and the potential for harvesting value in local economies. A brief review of existing biomass pretreatment technologies for cellulolytic hydrolysis at biorefineries is presented, followed by a discussion toward economically applying the underlying chemistries at reduced severity in light of capital and operational limitations of small-scale feedstock depots.

Renewable biomass represents a significant source of carbon-neutral domestic energy for the USA. A joint US Department of Agriculture and Department of Energy report estimated that more than 1 billion dry tons per year of biomass could sustainably be made available for conversion to renewable fuels and chemicals [1], potentially enabling the displacement of as much as 30% of current US petroleum consumption by 2030 [2]. While design of the biofuels supply chain is intended to integrate easily into existing transportation, handling and fuels infrastructure, significant investment is required to construct conversion facilities, and expand or adapt the associated feedstock and end-product handling and distribution systems. Securing financing for biofuels projects is challenging, in part because of risks inherent in the feedstock supply system [3]. The viability of conversion facilities depends on feedstock supply systems that ensure low-cost, high-volume feedstock supplies,

which meet the quality requirements of the conversion technology. Not all biomass is suitable for conversion to biofuels, biochemicals or biopower because biomass resources are inherently heterogeneous, variable in composition and quality, and can contain introduced soil and endogenous contaminants that are detrimental to downstream processing [4,5]. Currently, the feedstock supply risk is managed by relying only on single-species feedstocks that can be acquired within narrow delivered cost margins and meet minimum tolerable quality specifications. As such, just a small portion of the US biomass resource potential can be used for biofuel production.

Increasing the biomass resources that are suitable as feedstocks not only increases production capacity and helps achieve national renewable energy goals, but also has the potential to reduce feedstock supply risk and, thereby, spur industry investment. A number of approaches are being explored to enable greater

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FUTURE SCIENCE

Key terms

Lignocellulosic biomass: Highly abundant plant material comprised primarily of cellulose, hemicellulose and lignin. Examples include wood chips, wood waste, perennial grasses, agricultural residue or waste material that can be converted to biofuels or biochemicals.

Regionally distributed feedstock preprocessing depots: Local facilities that take biomass from the local area, preprocess it and densify it prior to shipping to a local terminal for distribution.

Chemical preconversion: Process whereby the structure and/or composition of biomass is modified via low-severity chemical reactions, in order to improve biomass feedstock commodity characteristics.

resource suitability, including optimizing logistics and preprocessing biomass early in the supply chain to take advantage of existing highly efficient, high-volume material handling systems. Adapting systems to be able to convert a variety of feedstocks is another option for reducing feedstock supply risk and capturing economies of scale benefits [6,7].

As biofuel is intended to offset consumption of imported, non-renewable energy commodity products, it is reasonable to consider how commoditization of biomass feedstocks can increase production capacity and investor confidence. As with other industries, transforming biomass into fungible, interchangeable feedstocks can maximize efficiency and productivity, through well-defined processes and minimization of overdesign [8]. For **lignocellulosic biomass**, some of the critical commodity parameters for ensuring large-scale utilization include aerobic stability, consistent specifications, transportability and interchangeability. There are other drivers for considering commoditization. Without feedstock costs, economies of scale favor large-scale biorefineries [9,10], but biomass density, variability, instability and limited harvest windows represent diseconomies of scale in feedstock supply systems. This is apparent in scale-up studies, as the economics of biofuels production have been shown to be potentially viable on a small scale from single lignocellulosic feedstocks [9–12], but the low bulk density of unprocessed biomass together with the risk of feedstock supply interruption leads to difficulty in obtaining financing, even if the technology is proven at the pilot scale [3].

Whether the feedstock supply systems of the future remain small and vertically integrated, include distributed preprocessing or develop into a number of feedstock commodity systems, there are some universal challenges that must be resolved to increase production capacity, including low bulk density, variability of feedstock properties and feedstock quality. A number of studies are addressing these challenges by preprocessing biomass at **regionally distributed feedstock preprocessing depots** to improve density and stability, and allow more economical transport to local biorefineries or distant terminals. However, the costs of preprocessing do not always justify the benefits gained.

This paper explores the potential of novel, low-severity **chemical preconversion** treatments, used in distributed preprocessing systems to increase use of biomass resources and enable industry expansion by improving costs and preprocessing effectiveness, and enabling the blending of resources and resource types while still meeting biorefinery specifications. We provide an overview of the regionally distributed feedstock preprocessing depot concept, and the types of preprocessing that could be used in the depot to improve quality and cost. The concept of chemical preconversion treatments that could be implemented in the depot to reduce processing and densification costs is introduced, from the standpoint of minimizing treatment severity in order to minimize processing costs. Following this, we describe the effects of various types of high-severity chemical treatments on lignocellulose, as learned through decades of development of high-severity pretreatment technologies designed to facilitate enzymatic conversion of the cellulose fraction of lignocellulose at scale-driven biorefineries. Finally, we discuss the pros and cons of applying the various chemistries and their requirements at reduced severities in distributed depots.

The role of low-severity chemical preconversion treatments in preprocessing depots

The economic viability of today's emerging biorefineries is tightly coupled to the resource availability within fairly small draw regions, and to the logistics cost of forestry and agricultural equipment and processes that were designed and optimized for other industries. Feedstocks currently undergo minimal densification and are transported directly from the production location to the biorefinery, where they are dried, stored, formatted and fed to the pretreatment process [13]. In forestry applications, harvest and collection of residues generally occurs by collection at the site and transportation to a staging area, where they are chipped and blown into a truck [14]. The chips are then transported to the conversion facility. In agricultural applications, residues are typically raked into windrows after harvest, field dried, densified by baling, and either stored in stacks at the field side and transported to the biorefinery as needed (just-in-time delivery) or directly transported by truck to the biorefinery [13]. Grinding of the feedstock (or further chipping for woody biomass) to prepare it for pretreatment occurs at the biorefinery. Because of the high water content associated with these feedstocks, aerobic stability and dry matter loss are a concern [13], and the low bulk density of the biomass leads to high transportation costs [3,15].

The regionally distributed feedstock preprocessing depot concept attempts to improve feedstock supply system efficiencies and capture costs savings by locating feedstock collection and preprocessing centers close to feedstock production locations **(Figure 1)** [13,16,17]. In the regionally distributed feedstock preprocessing

Figure 1. The regionally distributed feedstock preprocessing concept.
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concept, biomass from various sources is accumulated at collection centers, or 'depots', and preprocessed into aerobically stable, densified and flowable feedstocks. They are then economically transported to distant terminals where they can be blended, aggregated and/or amended to produce commodity feedstocks [13]. These depots may manage a single feedstock type or a variety of different feedstocks, which determines what preprocessing treatments will be implemented in a particular depot. Studies are underway to investigate the potential of depot-located mechanical preprocessing, chemical preconversion and formulation in enabling cost-effective densification, as well as improving composition and quality.

▪ Enabling cost-effective densification

Densification operations have been well studied for many biomass sources with regard to moisture, grinding requirements and significant processing parameters for pelletization [18,19]. An initial size reduction by chopping (agricultural residues) or chipping (woody materials) prepares the biomass for drying, before further grinding to particle size ranges better suited for pelletization. Specific energy requirements for this step increase with moisture content, feed rate and screen size [19]. For the second-stage grind, hammermills are typically employed for forage because they are low cost, can process many different types of biomass, are simple to operate and produce a wide range of particle sizes, which is desirable for pelleting. Energy consumption, geometric mean particle diameter and particle size distribution are important performance measures for this step [19]. While small mean particle diameters combined with wide particle size distributions are preferred for subsequent pelleting, energy costs of grinding increase significantly as particle size decreases [18]. Chemical preconversion chemistries that are particularly effective at disrupting or weakening the structure of the cell walls of the biomass should therefore lower the specific energy requirement for grinding to a given particle size, as well as allow increased throughput and/or finer grinds.

For the pelleting operation, biomass compressibility, pellet density, durability and specific energy consumption are important performance measures [18]. Higher compressibility leads to more dense pellets, which improves both durability and aerobic stability. For agricultural residues, the biomass preheat temperature, moisture content, applied pressure and particle size all play significant roles in determining pellet density. Pellet durability for a given biomass source is most affected by applied pressure and particle size. Specific energy consumption for pelleting of agricultural residues significantly increases with higher applied pressure and smaller particle sizes [18]. Chemical preconversion chemistries that reduce the mean particle size, while maintaining a wide particle size distribution after grinding, should lower the specific energy requirement for pelleting by improving the biomass compressibility. In addition, chemical preconversion chemistries that alter the chemical character of the biomass surface, or redistribute lignin or hemicellulose, could also improve binding properties for pelleting, potentially leading to lower biomass preheat temperatures and applied pressure requirements [16].

After pelleting or briquetting, the biomass is in a form that can be efficiently handled, stored and transported using existing grain technologies, lowering the costs of these operations. Further, pellets of a particular type of biomass, for example corn stover, could be shipped to distant terminals and blended with other sources of corn stover pellets from other locations, or aggregated with pellets produced from different biomass sources (e.g., switchgrass, hardwood and softwood) also shipped there from other locations. In this way, such a system could support the development of **commodity feedstock** specifications that can suit different biorefinery technologies or different markets.

▪ Improving composition & quality

Mechanical preprocessing treatments to improve composition and quality include fractional or sequential grinding to remove introduced ash (e.g., soil) [20], and separation of plant tissues that have varying or unfavorable physiological ash composition, such as separation of high-ash leaves and nodes from low-ash wheat straw stems [21]. Chemical preconversion treatments include low-severity chemical treatments applied to remove contaminants and toxins [22], to reduce grinding [23] and densification costs [16], and to improve the pretreatability and/or bioconvertability of the feedstock for the end user [23]. Enzymatic [24] and whole-cell biocatalyst treatments [25] could also be considered chemical preconversions. In addition, thermal treatments such as pyrolysis and torrefaction, which cause chemical modification of the biomass, could be considered a form of chemical preconversion, although not generally suited for downstream **bioconversion** processes [26]. Composition and quality can also be managed through feedstock formulation approaches, which include blending different sources of the same feedstock, aggregation of different feedstocks and amendment of raw or blended/aggregated with stabilizing agents or pretreatment chemicals [22].

Beyond improving composition and quality for local or regional biorefineries, an additional benefit of staging feedstocks in a depot is that the same preprocessing or preconversion methods can potentially be applied to improve the characteristics of the feedstock for use as a component of a biomass commodity feedstock [16]. In this scenario, the primary function of a depot would be to stabilize and densify the individual feedstocks for transportation to larger terminals that serve as the sources of single or aggregated commodity feedstocks for a variety of geographically distant end users [13]. In particular, the utilization of chemical preconversion treatments in the depot offers the potential to alter

the physical properties of biomass that make it expensive to format, handle, store and transport. Depending on the feedstock type, the chemistry used and its severity, chemical preconversion treatments also offer the potential for positively impacting the conversion characteristics of the feedstock once it reaches the biorefinery.

Existing biorefinery-scale pretreatments for biological conversion

Biological routes to fuels use enzymes from microorganisms to convert carbohydrates obtained from sugar, starch or lignocellulosic biomass through fermentation to ethanol, propanol or butanol. When lignocellulosics are the feedstock, the process must start with the hydrolysis of polysaccharide components to fermentable monomeric reducing sugars. Chemical preconversion methods are most likely to take advantage of the same chemistries utilized in the many precommercial chemical and enzymatic pretreatments described in the literature, which are being developed with the goal of improving the rate of cellulolytic hydrolysis to sugars at the biorefinery scale [27].

The goal of these biorefinery-scale pretreatments differs from the goal of chemical preconversion, in that pretreatments aim to improve the rate of cellulolytic hydrolysis via structural and chemical modification of the lignocellulose matrix. Because significant modifications to the matrix are required to achieve this, most chemical pretreatments are both capital and energy intensive. In addition, care must be taken to ensure that degradation products that can inhibit enzymes or fermentation organisms are not formed. Chemical routes that catalytically convert sugars into fuels must also utilize pretreatment and hydrolysis, but have a different set of inhibitory compounds that can interfere with catalyst performance. Hence, chemical preconversion technologies will likely not resemble their pretreatment technology counterparts, even though they employ the same chemical reactions. Reducing the severity of these chemical reactions to lower their energy and capital intensity will lead to less modification of the feedstock, but may perhaps modify the structure of the feedstock sufficiently to realize benefits to grinding, densification and composition in the feedstock supply chain, and commoditization of the feedstock.

Biochemical conversion processes can be sensitive to biomass structure, content and composition of polysaccharides and ash, and the presence of organic acids and phenolic inhibitors of enzymes and organisms [5]. Although outside the scope of this paper, thermochemical conversion processes, such as combustion for power production, gasification and pyrolysis, are known to be sensitive to ash content and ash composition [4], as

well as energy content. Few studies have considered the utilization of chemical treatments in distributed systems because the traditional purpose of chemical treatments (pretreatments) in biomass conversion has been to produce a pretreated substrate that can be efficiently hydrolyzed using fungal cellulase enzymes. Due to the recalcitrance of lignocellulose to conversion, these pretreatments typically have both high capital and high operating costs, minimizing the allowable feedstock cost, and thus, the scale of individual biorefineries.

As a basis for the reduced-severity preconversion treatments that are the focus of this paper, it is useful to review the industry standard chemical pretreatments used at the biorefinery and then consider the potential of locating decoupled chemistries earlier in the supply chain.

▪ Low-solids biorefinery-scale pretreatment technologies

Low-solids pretreatments (≤25 wt% solids) seek to dissolve and/or degrade one or more components of the lignocellulose matrix in order to increase enzyme accessibility to the remaining cellulose. Effective low-solids pretreatments extensively modify the structure of the matrix.

Acidic pretreatments

Acidic pretreatments increase cellulose digestibility by catalyzing the hydrolysis of intermonomer ether linkages in the hemicellulose and amorphous cellulose fractions of the lignocellulose matrix, as well as some linkages in the lignin fraction [28]. Requirements include heat, water and a source of protons. Acid-based methods are effective for hemicellulose solubilization and, to a smaller extent, lignin solubilization, but can generate sugar-derived inhibitors such as furfural and hydroxymethyl furfural. Depending on the catalyst used, acid pretreatments can be divided into three major groups: liquid hot water (LHW; autohydrolysis); acid hydrolysis with added acids; and the Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) process, where sulfite salts are added with mineral acids. These three pretreatments are described below.

LHW pretreatments

LHW pretreatments contact biomass with water at temperatures ranging from 160 to 240°C, and over lengths of time ranging from a few minutes up to an hour, with temperatures controlling the types of products formed and time controlling the amount of material solubilized [29]. The reactions that occur are described as autohydrolysis, which creates acid catalysts *in situ* due to the dissociative properties of water under these conditions, cleaving acetyl and uronic acid groups from

the hemicellulose to form acetic and other organic acids [30,31]. The autohydrolysis process is able to solubilize the more reactive hemicellulose and a portion of the lignin with minimum formation of inhibitors [32]. This fractionation disrupts the lignocellulosic matrix and generates more reactive cellulose fibers, which can be further hydrolyzed and saccharified by enzymes or additional acid treatments [33].

Acid pretreatments
Acid pretreatment involves the use of dilute or concentrated acids to hydrolyze a portion of the biomass. Hydrolysis of hemicellulose occurs first, solubilizing saccharides from the cell wall matrix and increasing porosity, which, in turn, improves enzymatic digestibility [34]. Dilute sulfuric acid is most commonly used due to its effectiveness and low cost, and has been used to pretreat a wide variety of biomass including woody [28,35,36] and herbaceous biomass [37,38]. Other acids have also been studied such as hydrochloric acid [39], phosphoric acid [40], nitric acid [41] and organic acids such as formic, maleic and fumaric acid [42,43]. Acid hydrolysis has historically been modeled as a homogeneous reaction in which acid catalyzes the breakdown of polysaccharides to oligomer or monomer sugars. The liberated sugar monomers can go on to form aldehydes and other degradation productions under harsh enough conditions. These aldehydes, principally furfural from pentoses and 5-hydroxymethyl furfural from hexose, are inhibitory to microbial fermentation [44].

SPORL pretreatments
SPORL is an acid pretreatment that has been reported to allow high amounts of saccharification for even recalcitrant woody material [45,46]. The pretreatment consists of a short chemical treatment of feedstock with a solution of a sulfite salt at 160–180°C and pH 2–4 for 30 min, followed by a size reduction. SPORL treatments produce readily digestible substrates through partial removal of hemicellulose and lignin, and an initial hydrolysis of cellulose. Due to the decreased acidity of SPORL, fermentation inhibitors are minimized as compared with dilute-acid treatments. Energy consumption for post-SPORL size reduction of wood chips was greatly reduced. Additionally, liquid-to-biomass ratios as low as three are possible, providing thermal energy savings.

Alkaline pretreatments
Dilute alkali pretreatment increases cellulose digestibility by disrupting the ester bonds cross-linking lignin and xylan. Alkali-based methods are effective for lignin solubilization but only partial hemicellulose solubilization. Depending on the catalyst used, low-solids alkaline

pretreatments can be divided into two major groups: treatment with sodium hydroxide (NaOH), potassium hydroxide, or calcium hydroxide (lime; $Ca(OH)_2$); and treatment with aqueous ammonia (NH_3). Dilute alkali treatment processes are generally very effective in the pretreatment of agricultural residues and herbaceous crops. Process conditions are relatively mild and generate few inhibitors, but reaction times can be long. NaOH, $Ca(OH)_2$ and NH_3 treatments are described below.

NaOH pretreatments
In the pulp and paper industry, NaOH has long been utilized together with sodium sulfite (alkaline sulfite pulping) or sodium sulfide (Kraft pulping) to produce cellulose pulp [47]. Although these technologies require substantial wastewater treatment, sodium sulfite pulping has recently been applied to corn stover [48]. Application of dilute NaOH alone results in the removal of most of the lignin and part of the hemicellulose, increased internal surface area and decreased degree of polymerization, leading to an increased reactivity of cellulose to subsequent hydrolysis [49]. Typical conditions for NaOH treatment are temperature ranging from 25 to 120°C, NaOH concentration from 0.5 to 2% (w/v), treatment time from hour to days, and solid-to-liquid ratios of 1:10–15 [50]. NaOH treatment is more effective on agricultural residues as compared with woody materials [35]. Although pretreatment with NaOH delignifies biomass and can realize significant yield improvements, high cost limits its use as a pretreatment for biofuels production.

$Ca(OH)_2$ pretreatments
$Ca(OH)_2$ as compared with NaOH and potassium hydroxide is cheaper and safer to handle. Typical $Ca(OH)_2$ loadings are 0.1–0.2 g $Ca(OH)_2$/g biomass. Approximately 5–15 g water/g biomass is required [51]. $Ca(OH)_2$ pretreatment can be performed at a variety of temperatures, ranging from 25 to 130°C, and the corresponding treatment times range from weeks at 25°C to hours at 130°C. Rates of conversion can be improved by providing more efficient heating via microwave irradiation [52]. Regardless of the temperature, $Ca(OH)_2$ treatment removes approximately 35% of lignin and approximately 100% of acetyl groups [53]. For low-lignin herbaceous materials this level of pretreatment is sufficient; however, for high-lignin woody materials the combined action of alkali and added molecular oxygen is required to solubilize significant portions of the lignin (~80%) and render the woody biomass digestible [53]. $Ca(OH)_2$ can be recovered for recycling by washing the biomass with water, which can then be saturated with CO_2 to form a calcium carbonate precipitate and fed to a kiln to regenerate the $Ca(OH)_2$ [54].

NH$_3$ pretreatments

Dilute aqueous NH$_3$-based processes have been proven to be highly effective in improving the enzyme digestibility of low-lignin feedstocks, such as corn stover. Biomass is soaked in 29.5 wt% aqueous NH$_3$ over an extended period (up to 10 days) at room temperature and atmospheric pressure with solid-to-liquid ratios of 1:10–15 [55]. Under these conditions, NH$_3$ in the aqueous solution reacts primarily with lignin, with little effect on carbohydrates in the biomass [55]. After soaking, the solids (composed of cellulose and hemicellulose) are separated by filtering, and washed with water to remove soluble lignin and residual NH$_3$. The Soaking in Aqueous Ammonia pretreatment removes up to 75% of the lignin, but retains nearly 100% of the glucan and 85% of the xylan. Due to the high cost of NH$_3$, an efficient NH$_3$ recovery process is required for this treatment.

Lignocellulose solvent pretreatments
Organosolvent pretreatments

Organosolv processes use an organic solvent, or mixtures of organic solvents with water, for removal of lignin before enzymatic hydrolysis of the cellulose fraction. In addition to lignin solubilization, hemicellulose is removed through acid-catalyzed hydrolysis, leading to improved enzymatic digestibility of the cellulose fraction. Common solvents for the process include ethanol, methanol, acetone and ethylene glycol. Temperatures used for the process can be as high as 200°C, but lower temperatures can be sufficient depending on the type of biomass and the use of an acidic catalyst [56]. The solvent itself can be an inhibitor for the enzymatic hydrolysis and fermentation step, and should instead be separated and recycled. Organosolv pretreatment allows for the fractionation of a relatively pure lignin, which has potential to produce other fuels or bioproducts.

Ionic liquid pretreatments

Ionic liquids (IL) are salts, typically composed of a small anion and a large organic cation, which exist as liquids at room temperature and have very low vapor pressures [57]. Processing of lignocellulosic biomass with ILs has garnered interest in the last decade due to the tunability of the solvent chemistry and ability to dissolve a wide variety of biomass types, including herbaceous materials and woods [58–60]. ILs can dissolve and decrystallize cellulose, which produces a highly reactive substrate for subsequent hydrolysis [61]. ILs have also been used with acid catalysts [62] and NH$_3$ treatments [63] to result in a near theoretical conversion of cellulose to glucose.

Supercritical CO$_2$ pretreatments

A supercritical fluid is a material in a state above its critical temperature and pressure, displaying unique properties intermediate to its gas and liquid properties. Supercritical fluids possess near-liquid density, while retaining gas-like transport properties of diffusivity and viscosity, which improve mass transfer. Kim and Hong found a treatment of supercritical CO$_2$ and steam provided an improvement in overall yield of sugar from wheat straw [64]. Recent work with high pressure (200 bar) CO$_2$-H$_2$O pretreatment of several different herbaceous biomass types demonstrated that a biphasic mixture comprised of a H$_2$O-rich liquid phase and a CO$_2$-rich supercritical phase coexists, and greatly aids in pretreatment [65]. This biphasic pretreatment produced glucose yields of 73–85% for wood, switchgrass and corn stover [65].

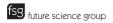### ▪ High-solids biorefinery-scale pretreatment technologies

As with low-solids pretreatments, high-solids pretreatments (>25 wt% solids) degrade one or more components of the lignocellulosic matrix in order to increase enzyme accessibility to the remaining cellulose, but this is achieved at significantly lower water content. In these pretreatments, the reactions take place at a liquid–solid interface, with the reaction between an insoluble polymer and a soluble moiety that either reacts with or solubilizes the biopolymer. Depending on the reactions and the aqueous solubility of the products, the water contained by the biomass can also allow dissolution of matrix components. Effective high-solids pretreatments extensively modify the structure of the matrix through depolymerization, oxidation and potentially redistribution of lignin.

NH$_3$-based pretreatments
Gaseous NH$_3$

Gaseous NH$_3$ has been used as a low-cost, low-quality pretreatment of biomass for animal feed purposes at the farm-scale for decades [66]. This process involves covering the agricultural residue with a tarp and pumping anhydrous NH$_3$ underneath it. Treatment occurs at atmospheric temperature for 4–8 weeks and 3% NH$_3$ loading, which partially solubilizes and relocates lignin [67]. A modification of this approach is the low-moisture anhydrous NH$_3$ pretreatment [68]. NH$_3$ gas is circulated through premoistened (30–70%) biomass to saturate it with NH$_3$ (12% of biomass weight), and is then sealed and incubated for 84 h at 80°C. This treatment is more effective at digesting polysaccharides than many other types of NH$_3$ treatments, including soaking in aqueous NH$_3$ and NH$_3$ recycle percolation. It also requires relatively low pressures, avoiding costly pressure vessels.

Ammonia Fiber Expansion pretreatments

Ammonia Fiber Expansion (AFEX™) is a moderate-pressure, low-water NH$_3$ pretreatment that has been

shown to be effective on a variety of grasses and grass residues. NH_3 loading is higher than other processes (0.7–2.0 g/g biomass). Temperatures range from 70 to 200°C and residence times of 5–30 min at a pressure of 150–300 psi [69]. After the desired pretreatment time, the pressure is released, vaporizing a portion of the NH_3; the remaining NH_3 must be evaporated and recompressed. Lignin is redistributed to the surface during the treatment and can act as a binder for subsequent densification [16]. AFEX produces few inhibitory compounds and sugar degradation relative to acid treatments [70].

Oxidative pretreatments
Ozone pretreatments
Ozone is a strong oxidizer that is highly soluble in water, decomposes to oxygen and has a strong affinity for carbon–carbon double bonds. These bonds are present throughout the structure of lignin while absent from polysaccharides and, therefore, ozone has been considered for pretreatment of a variety of agricultural residues and energy crops. A common method of ozone pretreatment is to sparge ozone through a fixed-bed reactor at a moisture content of 20–40% for 60–180 min. This can be performed at atmospheric pressure and temperature, although the ozonolysis reaction is exothermic [71]. During the process, lignin is depolymerized, removing approximately 50% of the total lignin from the biomass [72], and the pH of the biomass decreases to 2–3. An alkaline solution can prevent this pH drop, although this reduces the depolymerization of lignin [71].

Chlorine dioxide pretreatments
The strong oxidant chlorine dioxide (ClO_2) is used for both fiber delignification and bleaching in elemental chlorine-free pulp processing operations. In elemental chlorine-free bleaching stages, ClO_2 drives oxidative opening of lignin phenolic aromatic rings [73]; then, in extraction stages, an alkaline solution is used to mobilize and remove the oxidized lignin from the fiber. ClO_2 selectively oxidizes lignin with only moderately reduced cellulose fiber degree of polymerization and minimal sugar degradation [74]. When used as a pretreatment for enzymatic hydrolysis, ClO_2 pretreatment has been found to require extensive water washing to remove lignin, residual extractives and degradation products [75,201,202]. Due to its instability and toxicity, ClO_2 is rarely delivered as a gas to pulp processing plants, but is instead generated on-site from crystalline or aqueous sodium chlorate or chlorite, using a reducing agent such as hydrochloric or sulfuric acid, methanol, hydrogen peroxide or mixtures of those [76]. Sodium chlorate can be produced from sodium

chloride by electrolysis, simplifying supply logistics at the cost of increased capital equipment costs [77].

Acidic pretreatments
Nitrogen dioxide pretreatments
Nitrogen dioxide (NO_2) has been proposed as a high-solids chemical biomass treatment, but little experimental work has been done to develop the idea. In concept, absorption of NO_2 gas at moderate partial pressures by moist biomass could drive *in situ* formation of dilute nitric acid in the absorbed liquid phase. Dilute nitric acid has been studied extensively as an effective catalyst for acid hydrolysis of lignocellulosic biomass, including agricultural residues such as corn stover [41]. Treatment with NO_2 at ambient temperature might provide effective preconversion during biomass storage, depending on the kinetics of nitric acid-catalyzed hydrolysis. While the kinetics of dilute nitric acid hydrolysis have been studied at high temperatures for short residence times [41], hydrolysis kinetics have not been measured at low temperatures over long residence times.

Sulfur dioxide pretreatments
A substantial body of literature describes the use of sulfur dioxide (SO_2) gas impregnation to catalyze steam pretreatment of a wide variety of biomass types. Some biomass types that have shown significantly enhanced enzyme hydrolysis yields following SO_2-catalyzed steam treatment include aspen [78], corn stover [79] and sugarcane bagasse [80]. The presence of SO_2 during steam treatment is thought to enhance rates of lignin depolymerization, hemicellulose hydrolysis and lignin–carbohydrate complex disruption. The value of rapid decompression, or 'steam explosion,' and the need for delignification following SO_2-catalyzed steam treatment, can vary significantly with the type of biomass [81]. Feedstocks with high lignin content, such as softwood chips, require several water wash cycles following SO_2-catalyzed steam treatment to remove soluble lignin, hemicellulose and inhibitory compounds.

High-energy irradiation pretreatments
High-energy irradiation pretreatments have also been described at high-solids levels [5]. Yang *et al.* irradiated wheat straw with a Co-60 γ-source at a dose rate of 244.53 Gy/min, and studied the effects of the treatment on particle size distribution after grinding and on glucose yield from cellulolytic hydrolysis [82]. With irradiation treatments ranging from 3.4 to 40.9 h (doses of 50–600 kGy), they observed that increasing γ-irradiation doses improved grinding and slightly increased glucose yields versus untreated straw.

Bak *et al.* pretreated rice straw using electron beam irradiation, followed by hydrolysis with cellulase and β-glucosidase [83]. When rice straw was exposed to 80 kGy at 0.12 mA and 1 MeV, 52.1% glucose yield was observed, compared with 22.6% glucose yield with no treatment.

Biological pretreatments

Biological pretreatments are characterized by the use of whole-cell biocatalysts or added enzymes to effect degradation of primarily lignin, but with some losses of cellulose and hemicellulose in the case of whole-cell biocatalysts. Biological treatments modify the biomass more slowly than chemical treatments, but have the advantages that they operate at low temperatures and pressures, do not require corrosive chemicals and can often be performed in low-capital systems [25].

Whole-cell biological pretreatments

Fungal pretreatment of wood chips has been extensively studied to reduce the cost of pulping chemicals and energy inputs [84]. Fungal pretreatments perform essentially the same function as chemical pretreatments in that they remove hemicellulose and lignin, using a suite of enzymes. White-rot fungi are generally employed, as they typically degrade less cellulose than brown- or soft-rot fungi [84]. Choice of organism plays a major role in the effectiveness of whole-cell pretreatment of lignocellulose in pure cultures [85,86]. In inoculated nonsterile fungal pretreatment, Houghton *et al.* demonstrated that moisture content, nitrogen content and inoculum size were critically important contributing factors impacting the ability of the inoculated organism to effectively compete against the indigenous microflora [87].

In vitro enzymatic pretreatments

Because of the competitive environment and high potential for loss of sugar yields with whole-cell biocatalyst treatments, the potential for *in vitro* enzymatic pretreatments has also been studied, with mixed results. Anderson *et al.* pretreated Bermuda grass and Napier grass with ferulic acid esterase and observed that, while the esterase treatment increased the amount of free sugars released by cellulase enzymes, subsequent ethanol production was inhibited by phenolics released by the esterase [88]. Kuila *et al.* utilized a laccase enzyme to pretreat bamboo and achieved as much as 84% delignification, with good cellulolytic convertability after treatment [89]. Smith *et al.* tested the ability of endoxylanase treatment during ensiled storage of corn stover to reduce grinding energy requirements, and found that the treatment reduced the feedstock's specific grinding energy by 35% and increased the bin density after

grinding by 13%, compared with water-only controls [90]. Smith *et al.* tested the potential for endoxylanase pretreatment while in dry storage and observed endoxylanase activity on wheat arabinoxylan at water activities as low as 0.59, corresponding to water contents in corn stover, switchgrass and wheat straw of 7–13% on a wet basis [24]. Since filamentous fungi are typically not capable of activity below a water activity of approximately 0.7, the potential for loss of polysaccharides would be minimized in this system [91].

Considerations for implementation of low-severity pretreatment chemistries in a depot setting

Pretreatment technologies that can be economically employed at low severity in smaller scale systems will be better candidates for use as chemical preconversions. Chemical processes are generally cheaper to perform at large scales, such as a commercial biorefinery, as compared with a depot setting. Labor and overhead costs can be more expensive per ton of feedstock, as the same labor may be required to operate a 100 ton/day operation as a 1000 ton/day operation. Likewise, capital costs of chemical processes increase by factors ranging from 0.4- to 0.9-times the increase in capacity [92], meaning increasing the facility size by a factor of ten only increases the capital cost by factors ranging from 2.5- to 7.9-times. Thus, a chemical preconversion step should minimize labor and capital intensity to be competitive in a depot setting. It is therefore highly unlikely that the application of a particular low-severity pretreatment chemistry as a chemical preconversion treatment in a depot system will closely resemble high-severity pretreatment technologies that use the same chemistry in a large-scale biorefinery. Examples include long residence time treatments at reduced severity that do not require monitoring, treatments with no solid/liquid separation and low pressure treatments that can be performed in pits or under tarps rather than in reactors. Considerations for use of each preconversion chemistry described above are discussed below.

▪ Low-solids preconversions

If used as a chemical preconversion, structural modification of the lignocellulose matrix without significant removal of polysaccharides would be preferred. Low-solids pretreatment chemistries described above are shown in Table 1, with the expected chemical/structural effects desired for their reduced-severity use as chemical preconversion treatments. A significant concern for all low-solids preconversions is finding value for or disposing of the liquid stream, which can contain dissolved hemicellulose, lignin, organic acids and ash. In addition, since the biomass must be dried prior to grinding
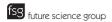
Table 1. Summary of low-solids pretreatment classes and their expected chemical/structural effects at reduced severity for use as a chemical preconversion.	
Pretreatment class	**Reduced-severity effect as chemical preconversion treatment**
Acidic	Partially depolymerize hemicellulose and some lignin depending on temperature and pH
Alkaline	Partially solubilize lignin and some hemicellulose depending on temperature and pH
Lignocellulose solvents: ▪ Organosolvents ▪ Ionic liquids ▪ Supercritical solvent	▪ Partially solubilize lignin and some hemicellulose ▪ Completely solubilize polysaccharides and lignin ▪ Solvent dependent; potentially any of the above

and pelleting, low-solids treatments will have a higher energy cost to remove the water and added capital cost for drying equipment. Decreased severity and reduced water use are two approaches to maintain value in a depot setting.

Of the available acidic low-solids preconversions, LHW treatment is the simplest and, thus, most likely as a preconversion process at a biomass depot. More efficient heating could be provided with microwaves rather than steam, potentially leading to capital cost savings. The treatment will not only produce more hydrolysable biomass, but will also leach out a portion of the inorganics, making a higher-quality feedstock. Use of reduced severity conditions (<160°C) will minimize carbohydrate solubilization and, if tuned properly, could reduce grinding and pelleting energy requirements.

Acid preconversions would undoubtedly be more effective at modifying the lignocellulosic matrix, but because of high acid recycling costs and occupational safety concerns with concentrated acids it is likely that only dilute-acid treatments would be considered for preconversion in a depot setting [93]. As with hot water, these treatments would likely be utilized at reduced severity to lower costs, and microwave heating could potentially lead to capital cost savings [52,94]. With the partial depolymerization of hemicellulose achieved at the reduced severity, the treated biomass would be easier to further reduce size and pellet. As with hot water treatment, the process must either be tuned to eliminate significant solubilization of the hemicellulose or alternative value-added uses for the resulting dissolved hemicellulose saccharides must be found. SPORL treatments are a type of acid preconversion that offer low-inhibitor formation and can be performed at milder conditions.

On the other end of the pH spectrum, low-severity dilute alkali treatments are advantageous in a depot setting due to lower capital cost, arising from lower corrosivity and simpler metallurgy requirements. Due to the mild processing conditions, low temperature and low pressure, none of the alkaline treatments listed above would require expensive pressure reactors, which reduces the overall capital cost compared with high-pressure treatments. The primary disadvantage of low-severity dilute alkali treatments is the fact that these

processes are all performed at a low solid-to-liquid ratio, and produce solid and liquid product streams. Finally, the ability to produce densified and stable products is critical to the feasibility of the distributed depot concept. Because lignin present in the biomass plays a significant role as a binder in densifying biomass, it would be necessary to carefully control the delignification reaction to minimize complete solubilization and separation during washing.

The organosolv, IL and supercritical fluid treatments would all require higher capital to recycle solvents or achieve high pressure, making these treatments less promising for preconversion in depots. Additional co-product from extracted lignin or other biomass moieties would make these treatments more economically realizable. However, it is the authors' opinion that significant additional work is needed before consideration for use in a depot setting.

▪ **High-solids preconversions**

High-solids treatments have the benefit over low-solids treatments in that they do not immediately generate a waste stream; however, some high-solids treatments require washing to remove reacted biomass fragments. High-solids pretreatments described above are shown in Table 2, with the expected chemical/structural effects desired for their reduced-severity use as chemical preconversion treatments.

Both the ammoniation and low-moisture anhydrous NH_3 technologies are low cost and simple enough to be well suited for utilization at a regional depot. NH_3 recovery would be necessary at a depot due to health and environmental concerns, as well as improving economics; hence, an efficient NH_3 recovery process is needed in order to make this approach viable. Delivered costs of anhydrous NH_3 vary widely across the USA, from less than US$640/ton in the south central states to more than $970/ton in the northwest, which can greatly impact chemical preconversions that consume either NH_3 or reagents produced from NH_3 [95].

AFEX pretreatment eliminates the need for a complicated NH_3 recovery design by directly transferring NH_3 from one reactor to the next. The pretreatment has been considered in decentralized facilities using a new

Table 2. Summary of high-solids pretreatment classes and their expected chemical/structural effects at reduced severity for use as a chemical preconversion.	
Pretreatment class	**Reduced-severity effect as chemical preconversion treatment**
Ammonia based	
Ammonia	Partially depolymerize hemicellulose and lignin
AFEX™	Partially depolymerize hemicellulose and lignin; redistribute lignin to surface of particles
Oxidative	
Ozone	Partially depolymerize lignin and hemicellulose; oxidize lignin
Chlorine dioxide	Moderate polysaccharide depolymerization, lignin oxidation
Acidic	
Nitrogen dioxide	Partially depolymerize hemicellulose
Sulfur dioxide	Partially depolymerize hemicellulose and some lignin
High-energy irradiation	
All types	Treatment dependent; also free radical crosslinking with ionizing radiation
Biological	
Whole cell	Partially depolymerize polysaccharides and lignin; dependent on organism and whether sterile or unsterile
In vitro enzymatic	Partially depolymerize hemicellulose and lignin; dependent on enzyme activity(ies) applied
AFEX: Ammonia Fiber Expansion.	

reactor system involving packed-bed reactors designed to accommodate the smaller scale of a depot [16,96]. Due to the high pressures required, compression costs must be considered when adapting the AFEX technology for a depot setting, but it remains a good candidate.

Both ozone and ClO_2 pretreatments are capable of degrading lignin with relatively low decomposition of carbohydrates [97,98]. In addition, the lack of extreme temperatures and pressures allows for a low capital cost preconversion suitable to regional processing. However, both chemical treatments create cost challenges due to storage and handling of the gases, and challenges in the handling and detoxification of product streams.

The gaseous acid pretreatment concept of NO_2 or SO_2 impregnation of moist biomass could possibly be used as a low-severity treatment to promote preconversion during storage at ambient temperature, provided reaction rates at low temperatures are fast enough to give significant hydrolysis. A recent study demonstrated a reduced severity SO_2 steam explosion treatment followed by pelletization and subsequent enzyme hydrolysis [99]. Treating moist biomass with a gas instead of dilute liquid acidic streams could benefit from the high surface area and porosity of ground biomass, thereby overcoming the limitations of liquid–solid contacting to achieve even concentration distribution in the biomass without mechanical mixing. The logistics of gas supply and storage are challenging to keep low cost. While some work of gas uptake by moist biomass has been done, additional kinetic experiments would be needed [78,100–102]. Additionally, water and air emissions from

the biomass treatment reactor would have to be carefully controlled, adding to the depot capital cost.

A much easier chemical system to handle would be the use of biological catalysts. Both low cost and low maintenance, they would seem to be good candidates for a depot setting, provided that there is sufficient land area around the depot to perform the treatments. Fungal treatments that degrade lignocellulose are obligately aerobic and, thus, a pile or windrow could not be covered, or if covered would need to be supplied with air (humidified to prevent drying). Careful monitoring of the ongoing treatments would be needed to ensure that inoculated organisms remain dominant during the treatments.

A preconversion technology that eliminates the need for chemical handling infrastructure altogether is high-energy irradiation preconversion. Unfortunately, these treatments are energy intensive and require rather large doses of radiation and/or very long exposure times depending on the substrate, and have shown only moderate improvements to glucose yields upon enzymatic hydrolysis [5]. Hence, for utilization at regional depots, safe and cost-effective sources of radiation would be critical. Although modifications at a depot are not necessarily limited to feedstock logistics benefits, in the case of high-energy irradiation treatments it would be important to achieve large reductions in required pretreatment severity at the biorefinery due to their high cost and energy intensity. In either case, safe operation would be a challenge in a rural depot.

Discussion

A national- or global-scale biorefining industry can potentially be enabled by dense, flowable, transportable biomass sources, provided that they can be economically densified and brought to a consistent composition and quality [3,15]. Such a product would allow a variety of uniformly formatted, individual feedstocks to be blended or aggregated at another site into commodity feedstocks to meet the needs of a single biorefinery, or many different biorefineries. Regional feedstock depots that individually stockpile, chemically preconvert, stabilize and densify nearby biomass offer potential for defining and achieving these commoditized biomass feedstocks to supply a growing biofuels industry.

Currently, all biomass pretreatment and conversion steps occur at the biorefinery, and feedstock cost is the overriding concern. This is primarily because of constraints on the biorefinery scale due to limited feedstock supply radius, and constraints on process efficiency due to feedstock variation. The net effect is that local feedstock supplies (and cost) are inextricably linked to the economic competitiveness of the biorefinery. Within this paradigm, increasing the feedstock cost is discouraged, and chemical preconversion is not favored unless the pretreatment is eliminated at the biorefinery. In this scenario, any chemical preconversion treatment – regardless of where it is performed – would need to be lower severity than full-severity pretreatment technologies, to ensure minimal added cost to the feedstock at the biorefinery gate. However, it is unlikely that low-severity treatments could open the cell wall structure sufficiently to eliminate the pretreatment at the centralized biorefinery. Further complicating the picture is that during subsequent drying, grinding and densification, hornification can potentially occur, leading to irreversible stiffening and shrinking of the fiber structure and a subsequent loss in enzymatic activity during cellulolytic hydrolysis at the biorefinery [103,104]. Little work has been done on regrinding of pellets to evaluate the effects of compaction of the biomass into pellets. Recent studies with enzymatically hydrolyzing ground or unground pellets have seen little to no reduction in cellulose digestibility, either with or without pretreatment, so regrinding and hornification effects may be less of a concern [99,105,106].

This paradigm can be shifted by decoupling feedstock quality and cost from the biorefinery to allow the biorefinery to focus its economics on producing bioproducts. This would allow greater feedstock availability, standardized feedstock specifications, improved economies of scale and, ultimately, commoditization of biomass feedstocks. This could be enabled by taking advantage of the three potential overarching benefits to the utilization of chemical preconversion in the distributed depot concept:

- Additional value capture at the local level;

- Production of a uniform commodity feedstock to enable a national- or global-scale biorefining industry;

- Reduced pretreatment costs at the centralized biorefinery when the feedstock is converted.

Realizing one or more of these benefits can lead to larger scale, more economical biofuel supplies. If the sale of high-value co-products or a higher value feedstock can generate additional revenue at the local level, there is greater incentive in building up the supply chain. Feedstock uniformity is highly desirable in a biorefinery. For single feedstock types supplied to small, local biorefineries, a blended commodity feedstock that balances variability to meet specifications for ash, carbohydrate and lignin compositional requirements can help to provide a more uniformly effective pretreatment to the biorefinery [107]. At a larger scale, which would require the use of a variety of feedstock types, it is certain that the different feedstocks will require different pretreatment severities. Aggregation of different feedstock types into commodity feedstocks that meet minimum ash, carbohydrate and lignin specifications, and also meet minimum biorefinery convertability requirements, would serve to ease the diseconomies of scale introduced by regional feedstock compositional variability and low bulk density.

Co-products are recognized as a necessity to improve the economics of a biorefinery [10,16,108]. The ability of a given chemical preconversion treatment to produce co-products in a regional depot will also play a role in the economics of applying chemical preconversion in the feedstock supply chain. In contrast to the biorefinery, where co-products can be produced at a large scale using the released sugars as feedstocks, co-products produced at a feedstock depot may need to have value without significant further processing or recovery costs. If the economics of smaller scale and low-severity modifications to biomass permit, co-products could include animal feed supplements from a number of pretreatment chemistries, including AFEX-treated biomass [16], hemicellulose oligomer syrups produced from various pretreatments [109] and hot water-extracted minerals from combustion or thermochemical-targeted biomass [110].

There are significantly greater potential benefits to utilizing chemical treatments to remove ash and sources of contaminants or toxins, while simultaneously reducing the cost of grinding and densification. Mild leaching or chemical preconversion treatments could be performed prior to densification to remove contaminants and/or to reduce the cost of grinding and densification through limited structural disruption of the lignocellulose matrix. Extensive structural modification of lignocellulose by chemical modification has been

shown to positively impact the performance and energy consumption of grinding and pelleting processes using several full-severity pretreatment technologies including AFEX [16], enzymes [90] and steam explosion [18]. Chemical modification of lignin or lignin redistribution can decrease wettability, which can help avoid biodeterioration during storage [203]. Leaching or releasing physiological ash from within cell walls can increase yields per ton of delivered feedstock [4]. Furthermore, ash or contaminant removal will allow tighter control on composition. Hence, chemical preconversion treatments used in this manner, combined with blending and aggregation at a terminal, serve to enable the development of commodity biomass feedstocks by addressing each of the critical commodity parameters for lignocellulosic biomass: aerobic stability, consistent specifications, transportability and interchangeability.

Different types of biomass will require different methods of preconversion because of their different chemical and structural characteristics (Table 3). Hardwoods and softwoods are high in lignin and, thus, susceptible to oxidative preconversion methods, whereas grasses with high acetyl content can be modified with mild acid or alkaline treatments. In addition, agricultural residues may also contain high ash content and, thus, a mild liquid treatment could also act as a leaching process. Depending on the feedstock and preconversion chemistry employed, low-severity chemical preconversions may be better suited to highly digestible feedstocks. In this case, a low-severity chemical preconversion may be sufficient to achieve high sugar yields from the biomass at the biorefinery without the need for additional pretreatment (added value at the biorefinery), while still producing a co-product in the depot (added value in the local economy). Thus, the technology present at each individual depot will be dependent on the landscape surrounding it, and more than one method of preconversion could potentially be applied. A diverse set of preconversion methods would be undesirable for producing a homogenous, commoditized feedstock at the depot due to increased capital costs. Hence, in regions where multiple types of biomass are available in large quantities, it may be more economical to have several depots, each dedicated to a single feedstock type. The set of preconversion methods that may develop will depend on the capability of the terminal to blend or aggregate homogenous feedstocks, and the number of different types of commodity feedstocks that the market desires.

Future perspective

While the economics of biofuels production may in some cases be viable at small scale from single or niche lignocellulosic feedstocks, this model is unlikely to be sufficient to support an integrated national- or global-scale bioeconomy, due to difficulty in obtaining financing. Without a cost-effective mechanism to supply consistent, stable and defined commodity feedstock(s) to end users deploying suitable technologies, the future landscape for renewable bioenergy may remain a collection of small, isolated regional industries relying

Table 3. Applicability of full-severity pretreatment technologies and reduced-severity chemical preconversion treatment chemistries to various types of feedstocks.		
Pretreatment/chemistry	**Applicable feedstock type**	
	Full-severity pretreatment	**Reduced-severity chemical preconversion**
Low-solids treatments		
Acidic:		
▪ Liquid hot water	HB	HB
▪ Dilute aqueous acid	HW, SW, HB, MSW	HB
▪ SPORL	HW, SW, HB, MSW	HB
Dilute aqueous alkaline:		
▪ Sodium hydroxide	HW, SW, HB, MSW	HB
▪ Calcium hydroxide	HW, SW, HB, MSW	HB
▪ Ammonia	HW, SW, HB, MSW	HB
Lignocellulose solvents:		
▪ Organosolvents	HW, SW, HB, MSW	HW, SW, HB, MSW
▪ Ionic liquids	HW, SW, HB, MSW	HW, SW, HB, MSW
▪ Supercritical solvent	HW, SW, HB, MSW	HW, SW, HB, MSW
High-solids treatments		
Ammonia based:		
▪ Ammonia	HB	HB
▪ AFEX™	HB	HB
Oxidative:		
▪ Ozone	HW, SW, HB	HW, SW, HB
▪ Chlorine dioxide	HW, SW, HB	HB
Acidic:		
▪ Nitrogen dioxide	HW, SW, HB, MSW	HW, SW, HB, MSW
▪ Sulfur dioxide	HW, SW, HB, MSW	HB
High-energy irradiation	HW, SW, HB, MSW	HW, SW, HB, MSW
Biological:		
▪ Whole cell	HW, SW, HB, MSW	HW, SW, HB, MSW
▪ Enzymatic	HW, SW, HB	HB
AFEX: Ammonia Fiber Expansion; HB: Herbaceous biomass (e.g., grasses, straw and stover); HW: Hardwoods; MSW: Municipal solid waste; SPORL: Sulfite pretreatment to overcome recalcitrance of lignocellulose; SW: Softwoods.		

on a narrow range of raw biomass sources, and always bearing the risk of feedstock supply interruption [111].

Whether this can economically occur in such a distributed supply system depends on a number of factors, some related to feedstock supply and commoditization, and others related to conversion characteristics for the end user. Commoditization of biomass feedstocks to support a large and integrated biofuels industry will require

consideration of these factors separately, emphasizing the importance of specifications and interchangeability of feedstocks for varying bioconversion processes, rather than a holistic approach that attempts to adapt the conversion process to the variety of available feedstocks. This necessarily includes focusing first on stabilizing and densifying the feedstocks to take advantage of existing handling technology, and reduced transportation

Executive summary

Background & the role of low-severity chemical preconversion treatments in preprocessing depots

- Renewable lignocellulosic biomass feedstocks have highly variable properties. Commoditization of these feedstocks through the establishment of regional feedstock depots that produce a dense, flowable and transportable biomass would assist the expansion of the biofuels industry by allowing greater transportation efficiency and minimization of conversion facility overdesign.
- A strategy postulated in this paper is to utilize mild leaching or chemical treatments, termed chemical preconversion, prior to densification to remove contaminants and/or to reduce the cost of grinding and densification through limited structural disruption of the lignocellulose matrix. This preconversion is most likely to take advantage of the same chemistries utilized in the many chemical and enzymatic pretreatment technologies described in literature.

Existing biorefinery-scale pretreatments for biological conversion

- Low-solids pretreatments are easily controlled and have high reaction rates, but produce waste streams, often produce fermentation inhibitors and are typically energy and capital intensive.
- High-solids chemical pretreatments are easily controlled and proceed at rates commensurate for use in biorefineries, but utilize toxic and highly reactive chemicals that must be safely managed, produce wash streams that often contain fermentation inhibitors, and are generally energy and capital intensive.
- High-solids pretreatments utilizing enzymes or whole-cell biocatalysts progress at slower rates and are less controllable than chemically catalyzed pretreatments, but are inherently lower capital.

Considerations for implementation of low-severity pretreatment chemistries in a depot setting
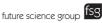
- A blended or aggregated commodity feedstock that balances the variability of composition, as well as convertibility of the individual component feedstocks, can allow a more uniformly effective pretreatment and hydrolysis at the centralized refinery.
- Since the biomass must be dried prior to grinding and pelleting, low-solids treatments will have a higher energy cost to remove the water and added capital cost for drying equipment. A significant concern for all low-solids preconversion treatments is finding value for or disposing of the liquid stream, which can contain dissolved hemicellulose, lignin, organic acids and ash.

Discussion

- A national- or global-scale biorefining industry can potentially be enabled by dense, flowable and transportable biomass sources, provided that they can be economically densified, and brought to a consistent composition and quality.
- Currently all biomass pretreatment and conversion steps occur at the biorefinery, inextricably linking only local feedstocks to the economic competitiveness of the biorefinery. This occurs because of constraints on biorefinery scale and process efficiency due to feedstock bulk density and compositional variations.
- This paradigm can be shifted by decoupling feedstock quality and cost from the biorefinery. This could be enabled by capturing valuable co-products at the local level, producing a uniform commodity feedstock and reducing the required pretreatment severity at the biorefinery.
- Chemical preconversion treatments, combined with blending and aggregation at distant terminals, enable the development of commodity biomass feedstocks by addressing each of the critical commodity parameters for lignocellulosic biomass: aerobic stability, consistent specifications, transportability and interchangeability.
- The technology present at each individual depot will be dependent on the landscape surrounding it, and more than one method of preconversion could potentially be applied. In regions where multiple types of biomass are available in large quantities, it may be more economical to have several depots, each dedicated to a single feedstock type.

Future perspective

- The establishment of regional feedstock depots that individually stockpile, chemically preconvert, stabilize and densify nearby biomass offers the potential for defining and achieving commoditized biomass feedstocks to supply a growing biofuels industry.
- Whether cost-effective supply of consistent, stable and defined feedstock(s) can economically be accomplished in a distributed supply system depends on factors related to feedstock supply and commoditization, and to conversion characteristics for the end user. Commoditization of biomass feedstocks to support a large and integrated biofuels industry will require consideration of these factors separately.
- Commoditizing the biomass supply and reducing feedstock supply risks will be necessary to enable sufficient biofuel production to meet the 2030 goal. Hence, feedstock cost is an important but perhaps not the overriding consideration. This circular argument has, and will continue to, limit development of a biofuels industry at a nationally significant scale.

and storage costs. Because chemical preconversion offers the potential for improving both feedstock characteristics and bioconversion characteristics, some bioconversion processes will ultimately be better performers, leading to the types of process improvements that have increased yields and reduced costs within the oil and petrochemical industry over the past century.

For a bioconversion end user, low feedstock cost is a critical consideration, given high capital and operating costs that are typical for production of fermentable sugars from cellulosic biomass. From the standpoint of commoditizing the biomass supply and reducing feedstock supply risks for a large biofuels industry – which will be necessary to enable sufficient biofuel production to meet the 2030 goal – feedstock cost is an important but perhaps not the overriding consideration. This circular argument has, and will continue to, limit development of a biofuels industry at a nationally significant scale. Chemical preconversion in a regional feedstock depot offers the potential for achieving commoditization to reduce feedstock supply risk and eliminate biorefinery overdesigning, while at the same time offering the potential for regionally produced co-products, capture of value by rural economies, and reduction of pretreatment-related capital and operating costs at the centralized biorefinery.

Disclaimer

Financial & competing interests disclosure

References

Papers of special note have been highlighted as:
- ■   of interest
- ■■   of considerable interest

1   US Department of Energy. *US Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry.* Oak Ridge National Laboratory, Oak Ridge, TN, USA (2011).

■■   **Summary of available biomass and biomass types in the USA.**

2   Foust TD, Wooley R, Sheehan J *et al. A National Laboratory Market and Technology Assessment of the 30 × 30 Scenario.* National Renewable Energy Laboratory, Golden, CO, USA (2007).

3   Thyfault C. The project finance market – is it open for biofuels? *Biofuels Int.* 6(4), 62–64 (2012).

■■   **Perspective article presenting the challenges with obtaining project financing in the developing biofuels industry.**

4   van Loo S, Koppejan J. *The Handbook of Biomass Combustion and Co-firing.* van Loo S, Koppejan J (Eds). Earthscan, Sterling, VA, USA (2008).

5   Zheng Y, Pan Z, Zhang R. Overview of biomass pretreatment for cellulosic ethanol production. *Int. J. Agric. Biol. Eng.* 2(3), 51–68 (2009).

6   Richard TL. Challenges in scaling up biofuels infrastructure. *Science* 329, 793–796 (2010).

■■   **Describes the challenges in obtaining sustainable feedstock supplies for a national-scale biorefining industry.**

7   Schouwenberg PP. Transforming wood pellets into a global commodity. In: *Global Wood Pellet Industry Market and Trade Study.* Cocchi M (Ed.). IEA Bioenergy Task 40: Sustainable International Bioenergy Trade, International Energy Agency, Paris, France, 170–175 (2011).

8   Wechsung A, Oldenburg J, Yu J, Polt A. Supporting chemical process design under uncertainty. *Braz. J. Chem. Eng.* 27(3), 451–460 (2010).

9   Ma S, Eckhoff SR. Economy of scale for biomass refineries: commodity vs. contract pricing, area utilization factor, and energy crops. *Trans. ASABE* 55(2), 599–607 (2012).

10   Humbird D, Davis, R, Tao L *et al. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to*

Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. National Renewable Energy Laboratory, Golden, CO, USA (2011).

11 Gnansounou E, Dauriat A. Techno-economic analysis of lignocellulosic ethanol: a review. *Bioresour. Technol.* 101, 4980–4991 (2010).

12 Mupondwa E, Li X, Tabil L *et al.* Technoeconomic analysis of wheat straw densification in the Canadian Prairie Province of Manitoba. *Bioresour. Technol.* 112, 355–363 (2012).

13 Hess JR, Kenney KL, Ovard LP, Searcy EM, Wright CT. *Commodity-Scale Production of an Infrastructure-Compatible Bulk Solid from Herbaceous Lignocellulosic Biomass.* Idaho National Laboratory, Idaho Falls, ID, USA (2009).

■ **Overview of biomass collection in a depot setting, including modeling efforts.**

14 Searcy EM, Hess JR. *Uniform-Format Feedstock Supply System: A Commodity-Scale Design to Produce an Infrastructure-Compatible Biocrude from Lignocellulosic Biomass.* Idaho National Laboratory, Idaho Falls, ID, USA (2010).

15 Sultana A, Kumar A. Optimal configuration and combination of multiple lignocellulosic biomass feedstocks delivery to a biorefinery. *Bioresour. Technol.* 102, 9947–9956 (2011).

16 Eranki PL, Bals BD, Dale BE. Advanced regional biomass processing depots: a key to the logistical challenges of the cellulosic biofuel industry. *Biofuels Bioprod. Biorefin.* 5, 621–630 (2011).

■ **Discusses the depot concept focusing on chemical preconversion, primarily Ammonia Fiber Expansion™ treatment.**

17 Carolan JE, Joshi SV, Dale BE. Technical and financial feasibility analysis of distributed bioprocessing using regional biomass pre-processing centers. *J. Agric. Food Indus. Org.* 5(2), Article 10 (2007).

18 Tabil L, Adapa P, Kashaninejad M. Biomass feedstock pre-processing – part 2: densification. In: *Biofuel's Engineering Process Technology.* dos Santos Bernardes MA (Ed.). InTech, New York, NY, USA (2011).

19 Tabil L, Adapa P, Kashaninejad M. Biomass feedstock pre-processing – part 1: pre-treatment. In: *Biofuel's Engineering Process Technology.* dos Santos Bernardes MA (Ed.). InTech, New York, NY, USA (2011).

20 Tumuluru JS, Hess JR, Boardman RD, Wright CT, Westover TL. Formulation, pretreatment, and densification options to improve biomass specifications for co-firing high percentages with coal. *Ind. Biotechnol.* 8(3), 113–132 (2012).

■■ **Strong review of thermochemical processing and considerations for a depot setting.**

21 Hess JR, Thompson DN, Hoskinson RL, Shaw PG, Grant DR. Physical separation of straw stem components to reduce silica. *Appl. Biochem. Biotechnol.* 105(1–3), 43–51 (2003).

22 Idaho National Laboratory. *Densification Workshop: Transforming Raw Biomass to Feedstock – Summary Report.* Kenney KL, Ovard LP (Eds). Idaho National Laboratory, Idaho Falls, ID, USA (2012).

■ **The results of a feedstock workshop held by the US Department of Energy, Office of the Biomass Program and hosted by the Idaho National Laboratory.**

23 Zhu JY, Pan XJ, Zalesny RS Jr. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies and recalcitrance. *Appl. Microbiol. Biotechnol.* 87(3), 847–857 (2010).

■■ **Discusses energy consumption for pretreatment, pretreatment energy efficiency, pretreatment technologies and quantification of recalcitrance for woody biomass.**

24 Smith WA, Thompson DN, Thompson VS, Radtke CW, Carter B. Assessment of xylanase activity in dry storage as a potential method of reducing feedstock cost. *Appl. Biochem. Biotechnol.* 154(1–3), 287–301 (2009).

25 Tian X-F, Fang Z, Guo F. Impact and prospective of fungal pre-treatment of lignocellulosic biomass for enzymatic hydrolysis. *Biofuels Bioprod. Biorefin.* 6, 335–350 (2012).

26 Tumuluru JS, Sokhansanj S, Hess JR, Wright CT, Boardman RD. A review on biomass torrefaction process and product properties for energy applications. *Indus. Biotechnol.* 7(5), 384–401 (2011).

27 Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresour. Technol.* 101(13), 4851–4861 (2010).

■ **Recent review on pretreatment technologies, including their properties and estimated capital cost.**

28 Wyman CE, Dale BE, Elander RT *et al.* Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. *Biotechnol. Prog.* 25(2), 333–339 (2009).

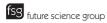29 Yu Y, Wu HW. Understanding the primary liquid products of cellulose hydrolysis in hot-compressed water at various reaction temperatures. *Energy Fuels* 24, 1963–1971 (2010).

30 Ingram T, Rogalinski T, Bockemuhl V, Antranikian G, Brunner G. Semi-continuous liquid hot water pretreatment of rye straw. *J. Supercrit. Fluids* 48(3), 238–246 (2009).

31 Pu Y, Treasure T, Gonzalez R, Venditti R, Jameel H. Autohydrolysis pretreatment of mixed hardwoods to extract value prior to combustion. *BioResources* 6(4), 4856–4870 (2011).

32 Perez JA, Ballesteros I, Ballesteros M, Saez F, Negro MJ, Manzanares P. Optimizing liquid hot water pretreatment conditions to enhance sugar recovery from wheat straw for fuel-ethanol production. *Fuel* 87(17–18), 3640–3647 (2008).

33 Yu Q, Zhuang XS, Yuan ZH *et al.* Two-step liquid hot water pretreatment of *Eucalyptus grandis* to enhance sugar recovery and enzymatic digestibility of cellulose. *Bioresour. Technol.* 101(13), 4895–4899 (2010).

34 Silverstein RA, Chen Y, Sharma-Shivappa RR, Boyette MD, Osborne J. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresour. Technol.* 98(16), 3000–3011 (2007).

35 Kumar R, Wyman CE. Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnol. Prog.* 25, 302–314 (2009).

36 Zhang C, Runge T. Fractionating pentosans and hexosans in hybrid poplar. *Ind. Eng. Chem. Res.* 51(1), 133–139 (2011).

37 Digman MF, Shinners KJ, Muck RE, Dien BS. Full-scale on-farm pretreatment of perennial grasses with dilute acid for fuel ethanol production. *Bioenergy Res.* 3(4), 335–341 (2010).

38 Yang B, Wyman CE. Dilute acid and autohydrolysis pretreatment. In: *Biofuels: Methods and Protocols.* Mielenz JR (Ed.). Humana Press, New York, NY, USA, 103–114 (2009).

39 Herrera A, Tellez-Luis SJ, Ramirez JA, Vazquez M. Production of xylose from sorghum straw using hydrochloric acid. *J. Cereal Sci.* 37(3), 267–274 (2003).

40 Boonmanumsin P, Treeboobpha S, Jeamjumnunja K, Luengnaruemitchai A, Chaisuwan T, Wongkasemjit S. Release of monomeric sugars from *Miscanthus sinensis* by microwave-assisted ammonia and phosphoric acid treatments. *Bioresour. Technol.* 103(1), 425–431 (2012).

41   Zhang R, Lu XB, Liu Y, Wang XY, Zhang ST. Kinetic study of dilute nitric acid treatment of corn stover at relatively high temperature. *Chem. Eng. Technol.* 34(3), 409–414 (2011).

42   Kootstra AMJ, Beeftink HH, Scott EL, Sanders JPM. Comparison of dilute mineral and organic acid pretreatment for enzymatic hydrolysis of wheat straw. *Biochem. Eng. J.* 46(2), 126–131 (2009).

43   Sindhu R, Binod P, Satyanagalakshmi K *et al.* Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol. *Appl. Biochem. Biotechnol.* 162(8), 2313–2323 (2010).

44   Mosier N, Wyman C, Dale B *et al.* Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* 96(6), 673–686 (2005).

45   Zhang C, Zhu JY, Gleisner R, Sessions J. Fractionation of forest residues of Douglas-fir for fermentable sugar production by SPORL pretreatment. *Bioenerg. Res.* 5(4), 978–988 (2012).

46   Shuai L, Yang Q, Zhu JY *et al.* Comparative study of SPORL and dilute-acid pretreatments of spruce for cellulosic ethanol production. *Bioresour. Technol.* 101(9), 3106–3114 (2010).

47   Gellerstedt G. Chemistry of chemical pulping. In: *Pulp and Paper Chemistry and Technology: Pulping Chemistry and Technology.* Ek M, Gellerstedt G, Henriksson G (Eds). Walter de Gruyter, Berlin, Germany, 91–120 (2009).

48   Latibari AJ, Hossein MA, Hosseinpour R. Application of alkaline sulfite pulping on corn stalks. *BioResources* 6(1), 48–58 (2011).

49   Wang Z, Keshwani DR, Redding AP, Cheng JJ. Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass. *Bioresour. Technol.* 101(10), 3583–3585 (2010).

50   Xu J, Cheng JJ, Sharma-Shivappa RR, Burns J. Sodium hydroxide pretreatment of switchgrass for ethanol production. *Energy Fuels* 24, 2113–2119 (2010).

51   Sierra R, Granda CB, Holtzapple M. Lime pretreatment. In: *Biofuels: Methods and Protocols.* Mielenz JR (Ed.). Humana Press, New York, NY, USA, 115–124 (2009).

52   Saha BC, Biswas A, Cotta MA. Microwave pretreatment enzymatic saccharification and fermentation of wheat straw to ethanol. *J. Biobased Mater. Bioenergy* 2, 210–217 (2008).

53   Granda CB, Holtzapple MT, Luce G, Searcy K, Mamrosh L. Carboxylate platform: the mixed process part 2: process economics. *Appl. Biochem. Biotechnol.* 153–156, 537–554 (2009).

54   Rabelo SC, Filho RM, Costa AC. Lime pretreatment of sugarcane bagasse for bioethanol production. *Appl. Biochem. Biotechnol.* 153–156, 139–150 (2009).

55   Kim TH, Lee YY. Pretreatment of corn stover by soaking in aqueous ammonia at moderate temperature. *Appl. Biochem. Biotechnol.* 136–140, 81–92 (2007).

56   Sun Y, Cheng JY. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour. Technol.* 83(1), 1–11 (2002).

57   van Rantwijk F, Lau RM, Sheldon RA. Biocatalytic transformations in ionic liquids. *Trends Biotechnol.* 21(3), 131–138 (2003).

58   Caes BR, Binder JB, Blank JJ, Raines RT. Separable fluorous ionic liquids for the dissolution and saccharification of cellulose. *Green Chem.* 13(10), 2719–2722 (2011).

59   Vancov T, Alston AS, Brown T, McIntosh S. Use of ionic liquids in converting lignocellulosic material to biofuels. *Renewable Energy* 45, 1–6 (2012).

60   Wang Y, Radosevich M, Hayes D, Labbe N. Compatible ionic liquid-cellulases system for hydrolysis of lignocellulosic biomass. *Biotechnol. Bioeng.* 108(5), 1042–1048 (2011).

61   Dadi AP, Schall CA, Varanasi S. Mitigation of cellulose recalcitrance to enzymatic hydrolysis by ionic liquid pretreatment. *Appl. Biochem. Biotechnol.* 137, 407–421 (2007).

62   Zhang ZH, Wang WQ, Liu XY *et al.* Kinetic study of acid-catalyzed cellulose hydrolysis in 1-butyl-3-methylimidazolium chloride. *Bioresour. Technol.* 112, 151–155 (2012).

63   Nguyen TAD, Kim KR, Han SJ *et al.* Pretreatment of rice straw with ammonia and ionic liquid for lignocellulose conversion to fermentable sugars. *Bioresour. Technol.* 101(19), 7432–7438 (2010).

64   Kim KH, Hong J. Supercritical CO_2 pretreatment of lignocellulose enhances enzymatic cellulose hydrolysis. *Bioresour. Technol.* 77(2), 139–144 (2001).

65   Luterbacher JS, Tester JW, Walker LP. Two-temperature stage biphasic CO_2-H_2O pretreatment of lignocellulosic biomass at high solid loadings. *Biotechnol. Bioeng.* 109(6), 1499–1507 (2012).

66   Van Soest PJ. Rice straw, the role of silica and treatments to improve quality. *Anim. Feed Sci. Technol.* 130, 137–171 (2006).

67   Krueger NA, Adesogan AT, Staples CR *et al.* Effect of method of applying fibrolytic enzymes or ammonia to Bermudagrass hay on feed intake, digestion, and growth of beef steers. *J. Anim. Sci.* 86, 882–889 (2007).

68   Yoo CG, Nghiem NP, Hicks KB, Kim TH. Pretreatment of corn stover using low-moisture anhydrous ammonia (LMAA) process. *Bioresour. Technol.* 102, 10028–10034 (2011).

69   Sousa LC, Chundawat SPS, Balan V, Dale BE. 'Cradle-to-grave' assessment of existing lignocellulose pretreatment technologies. *Curr. Opin. Biotechnol.* 20, 339–347 (2009).

70   Chundawat SPS, Vismeh R, Sharma LN *et al.* Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX™) and dilute acid based pretreatments. *Bioresour. Technol.* 101, 8429–8438 (2010).

71   Garcia-Cubero MT, Gonzalez-Benito G, Indacoechea I, Coca M, Bolado S. Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rice straw. *Bioresour. Technol.* 100, 1608–1613 (2009).

72   Kojima Y, Yoon SL. Improved enzymatic hydrolysis of waste paper by ozone pretreatment. *J. Mater. Cycles Waste Manage.* 10, 134–139 (2008).

73   Kumar P, Nikakhtari H, Nemati M, Hill G, Headley J. Oxidation of phenol in a bioremediation medium using chlorine dioxide. *J. Chem. Technol. Biotechnol.* 85, 720–725 (2010).

74   Yanagisawa M, Isogai A. Size exclusion chromatographic and UV-VIS absorption analyses of unbleached and bleached softwood kraft pulps using LiCl/1,3-dimethyl-2-imidazolidinone as a solvent. *Holzforschung* 61(3), 236–241 (2007).

75   Kuhad RC, Gupta R, Khasa YP, Singh A. Bioethanol production from *Lantana camara* (red sage): pretreatment, saccharification and fermentation. *Bioresour. Technol.* 101(21), 8348–8354 (2010).

76   Jin RY, Hu S-Q, Zhang Y-G, Bo T. Concentration-dependence of the explosion characteristics of chlorine dioxide gas. *J. Hazard. Mater.* 166, 842–847 (2009).

77   Pillai KC, Kwon TO, Park BB, Moon IS. Studies on process parameters for chlorine dioxide production using IrO_2 anode in an un-divided electrochemical cell. *J. Hazard. Mater.* 164, 812–819 (2009).

78   De Bari I, Nanna F, Braccio G. SO_2-catalyzed steam fractionation of aspen chips for bioethanol production: optimization of the catalyst impregnation. *Ind. Eng. Chem. Res.* 46(23), 7711–7720 (2007).

79   Ouyang J, Ma R, Huang W, Li X, Chen M, Yong Q. Enhanced saccharification of SO_2 catalyzed steam-exploded corn stover by

polyethylene glycol addition. *Biomass Bioenergy* 35, 2035–2058 (2011).

80   Carrasco C, Baudel HM, Sendelius J *et al.* SO₂-catalyzed steam pretreatment and fermentation of enzymatically hydrolyzed sugarcane bagasse. *Enzym. Microb. Technol.* 46, 64–73 (2010).

81   Ewanick SM, Bura R, Saddler JN. Acid-catalyzed steam pretreatment of lodgepole pine and subsequent enzymatic hydrolysis and fermentation to ethanol. *Biotechnol. Bioeng.* 98(4), 737–746 (2007).

82   Yang CP, Shen ZQ, Yu GC *et al.* Effect and aftereffect of gamma radiation pretreatment on enzymatic hydrolysis of wheat straw. *Bioresour. Technol.* 99(14), 6240–6245 (2008).

83   Bak JS, Ko JK, Han YH *et al.* Improved enzymatic hydrolysis yield of rice straw using electron beam irradiation pretreatment. *Bioresour. Technol.* 100(3), 1285–1290 (2009).

84   Singh P, Sulaiman O, Hashim R, Rupani PF, Peng LC. Biopulping of lignocellulosic material using different fungal species: a review. *Rev. Environ. Sci. Biotechnol.* 9, 141–151 (2010).

85   Shi J, Sharma-Shivappa RR, Chinn M, Howell N. Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production. *Biomass Bioenergy* 33, 88–96 (2009).

86   Lee J-W, Gwak K-S, Park J-Y *et al.* Biological pretreatment of softwood *Pinus densiflora* by three white rot fungi. *J. Microbiol.* 45(6), 485–491 (2007).

87   Houghton TP, Thompson DN, Hess JR *et al.* Fungal upgrading of wheat straw for straw-thermoplastics production. *Appl. Biochem. Biotechnol.* 113(1–3), 71–93 (2004).

88   Anderson WF, Peterson J, Akin DE, Morrison WH III. Enzyme pretreatment of grass lignocellulose for potential high-value co-products and an improved fermentable substrate. *Appl. Biochem. Biotechnol.* 121–124, 303–310 (2005).

89   Kuila A, Mukhopadhyay M, Tuli DK, Banerjee R. Accessibility of enzymatically delignified bambusa bambos for efficient hydrolysis at minimum cellulase loading: an optimization study. *Enzym. Res.* 805795 (2011).

90   Smith WA, Williams CL, Thompson DN, Thompson VS. Effect of endoxylanase treatment during storage on the grinding energy and storage density of corn stover feedstock. Paper #14143. Presented at: *32nd Symposium on Biotechnology for Fuels and Chemicals*. Clearwater Beach, FL, USA, 19–22 April 2010.

91   Roos YH. Water activity and plasticization. In: *Food Shelf Life Stability: Chemical, Biochemical, and Microbiological Changes*. Akivah N, Eskin M, Robinson DS (Eds). CRC Press, Boca Raton, FL, USA, 3–36 (2001).

92   Gallagher PW, Brubaker H, Shapouri H. Plant size: capital cost relationships in the dry mill ethanol industry. *Biomass Bioenergy* 28, 565–571 (2005).

93   Radillo JJV, Ruiz-Lopez MA, Macias RR, Ramirez LB, Garcia-Lopez PM, Toral F. Fermentable sugars from *Lupinus rotundiflorus* biomass by concentrated hydrochloric acid hydrolysis. *BioResources* 6(1), 344–355 (2011).

94   Ma H, Liu WW, Chen X *et al.* Enhanced enzymatic saccharification of rice straw by microwave pretreatment. *Bioresour. Technol.* 100(3), 1279–1284 (2009).

95   US Department of Agriculture. *Agricultural Prices, April 30, 2012*. National Agricultural Statistics Service, Agricultural Statistics Board, Washington, DC, USA (2012).

96   Bals BD, Dale BE. Developing a model for assessing biomass processing technologies within a local biomass processing depot. *Bioresour. Technol.* 106, 161–169 (2012).

97   Lee JM, Jameel H, Venditti RA. Effect of ozone and autohydrolysis pretreatments on enzymatic digestibility of coastal Bermuda grass. *BioResources* 5, 1084–1101 (2010).

98   Gupta R, Yogender PK, Ramesh CK. Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. *Carbohydr. Polym.* 84, 1103–1109 (2011).

99   Kumar L, Tooyserkani Z, Sokhansanj S, Saddler J. Does densification influence the steam pretreatment and enzymatic hydrolysis of softwoods to sugars? *Bioresour. Technol.* 121, 190–198 (2012).

100   Yabushita A, Enami S, Sakamoto Y, Kawasaki M, Hoffman MR, Colussi AJ. Anion-catalyzed dissolution of NO₂ on aqueous microdroplets. *J. Phys. Chem. A* 113, 4844–4848 (2009).

101   Monavari S, Galbe M, Zacchi G. Impact of impregnation time and chip size on sugar yield in pretreatment of softwood for ethanol production. *Bioresour. Technol.* 100, 6312–6316 (2009).

102   Mabee WE, Gregg DJ, Arato C *et al.* Updates on softwood-to-ethanol process development. *Appl. Biochem. Biotechnol.* 129–132, 55–70 (2006).

103   Diniz JMBF, Gil MH, Castro JAAM. Hornification – its origin and interpretation in wood pulps. *Wood Sci. Technol.* 37, 489–494 (2004).

104   Luo XL, Zhu JY, Gleisner R, Zhan HY. Effects of wet-pressing-induced fiber hornification on enzymatic saccharification of lignocelluloses. *Cellulose* 18, 1055–1062 (2011).

105   Rijal B, Igathinathane C, Karki B, Yu M, Pryor SW. Combined effect of pelleting and pretreatment on enzymatic hydrolysis of switchgrass. *Bioresour. Technol.* 116, 36–41 (2012).

106   Ray AE, Hoover AN, Nagle NJ, Chen X, Gresham GL. Effect of pelleting on the recalcitrance and bioconversion of dilute-acid pretreated corn stover under low- and high-solids conditions. *Biofuels* 4(3), 271–284 (2013).

107   Kenney KL, Smith WA, Gresham GL, Westover TL. Understanding biomass feedstock variability. *Biofuels* 4(1), 111–127 (2013).

108   Yang B, Wyman CE. Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels Bioprod. Bioref.* 2, 26–40 (2008).

109   Larsen J, Østergaard Haven M, Thirup L. Inbicon makes lignocellulosic ethanol a commercial reality. *Biomass Bioenergy* 46, 36–45 (2012).

110   Runge T, Wipperfurth P, Zhang C. Improving biomass combustion quality using a liquid hot water treatment. *Biofuels* 4(1), 73–83 (2013).

111   Miranowski J, Khanna M, Hess JR. Economics of feedstock production, harvest, storage, and transport. In*: Sustainable Alternative Fuel Feedstock Opportunities, Challenges and Roadmaps for Six US Regions, Proceedings of the Sustainable Feedstocks for Advance Biofuels Workshop, Atlanta, GA, USA, 28–30 September 2010 (Chapter 11)*. Braun R, Karlen D, Johnson D (Eds). Soil and Water Conservation Society, Ankeny, IA, USA, 177–192 (2011).

▪ **Patents**

201   Nguyen XN: US7666637 (2010).

202   Sannigrahi P, Rasgauskas AJ, Miller SJ: US0040413 (2012).

203   Dale B, Ritchie B, Marshall D: US0300269 (2011).